[0055] The geometric relationship between the electrodes may also vary. For example, the electrodes may be aligned in any order capable of generating the desired detection current. Likewise, each electrode may be positioned at any desired angle to the flow of the test sample. In the embodiments shown in **FIGS. 3-5**, the substrates **40** and **80** are laminated such that the working electrode **42** is positioned adjacent to the reference electrode **46**, the reference electrode **46** is positioned adjacent to the calibration working electrode **44**, and the calibration working electrode **44** is positioned adjacent to the counter electrode **48**. Moreover, each electrode is also positioned perpendicular to the flow of the test sample. Of course, various other electrode configuration and/or geometric relationships may be utilized in the present invention.

[0056] Regardless of the particular configuration of the electrodes, the present inventors have discovered that the use of separate substrates **40** and **80** may substantially reduce background interference. Specifically, the use of separate substrates allows the working electrodes **42** and **44** to be treated with affinity reagents without regard to potential contamination of the reference and counter electrodes **46** and **48** that would otherwise cause background interference. Once the treatment process is complete, the reference and counter electrodes **46** and **48** may then be placed in the desired location. Although not required, the laminated substrates **40** and **80** may be laminated in the desired position using any well-sealing sealing technique, such as by adhesively attaching the substrates **40** and **80** with glue, double-sided tape, and so forth. The extent that the substrates **40** and **80** are laminated together may vary as desired. For example, in some embodiments, the entire periphery of the substrates **40** and **80** are adhesively attached.

[0057] Various embodiments of detection the presence of an analyte within a test sample using the assay device **20** of **FIG. 4** or **5** will now be described in more detail. It should be understood, however, that the embodiments discussed below are only exemplary, and that other detection techniques and assay device configurations are also contemplated by the present invention. To initiate the detection of an analyte within a test sample, a user may simply apply the test sample to the sample pad **21** through which it may then travel. From the sample pad **21**, the test sample then travels to the conjugate pad **22** where any analyte within the test sample mixes with and attaches to a redox label. In one embodiment, for instance, the label is horseradish peroxidase (HRP) and the analyte of interest is glucose. Because the conjugate pad **22** is in fluid communication with the sample channel **14**, the labeled analyte may migrate from the conjugate pad **22** to the channel **14** through which it travels for the desired amount of time until it reaches the detection working electrode **42**, where the labeled analyte binds to the specific binding capture ligand and reacts with a redox mediator. In one embodiment, for example, the analyte is reacted as follows:

Analyte (reduced form)+Redox Mediator (oxidized form)→Analyte (oxidized form)+Redox Mediator (reduced form)

[0058] In addition, non-specific binding may be monitored and corrected using the optional calibration working electrode **44**. It is intended that the amount of non-analyte materials that bind to the calibration working electrode **44** will be similar to the amount of non-analyte material that non-specifically binds to the detection working electrode **42**. Thus, in this manner, the background signal due to non-specific binding may be compensated. In one embodiment, for example, the non-analyte biological materials (abbreviated "NAB") are reacted as follows:

NAB (reduced form)+Redox Mediator (oxidized form)→NAB (oxidized form)+Redox Mediator (reduced form)

[0059] Detection techniques, such as amperometric, coulometric, voltammetric, etc., may then be used to detect the analyte. A further description of such electrochemical detection techniques is described in *Electrochemical Methods*, A. J. Bard and L. R. Faulner, John Wiley & Sons (1980). In one embodiment, for example, a potentiostat or reader may apply a potential difference between the detection working electrode **42** and counter electrode **46**. When the potential difference is applied, the amount of the oxidized form of the redox mediator at the counter electrode **46** and the potential difference is sufficient to cause diffusion limited electro-oxidation of the reduced form of the redox mediator at the surface of the detection working electrode **42**. The magnitude of the required potential is thus dependent on the redox mediator. Namely, the potential is typically large enough to drive the electrochemical reaction to or near completion, but not large enough to induce significant electrochemical reaction of interferents, such as urate, ascorbate, and acetaminophen, that may affect the current measurements. Similarly, the potential difference may also be supplied between the optional calibration working electrode **44** and counter electrode **46**. When the potential difference is applied, diffusion limited electro-oxidation of the reduced form of the redox mediator occurs at the surface of the calibration working electrode **44**.

[0060] Generally, the detection and calibration working electrodes **42** and **44** simultaneously generate a respective signal from a single measurement of a sample. The simultaneously generated signals are averaged by a processing circuit, such as a multi-channel potentiostat. Multi-channel potentiostats are well known in the art, and are described, for instance, in U.S. Pat. No. 5,672,256 to Yee, which is incorporated herein in its entirety by reference thereto for all purposes. Each channel of a multi-channel potentiostat may function as a potentiostat, and thus may be associated with its own reference and/or counter electrode, or may share reference and/or counter electrodes. One suitable example of a multi-channel potentiostat that may be used in the present invention is commercially available under the name "MSTAT" from Arbin Instruments, Inc. of College Station, Tex. Once detected, the current measured at the detection working electrode **42** is calibrated by the current measured at the calibration working electrode **44** to obtain a calibrated current reading that may be correlated to the concentration of analyte in the sample. The correlation may result from predetermined empirical data or an algorithm, as is well known in the art. If desired, the generated current and analyte concentration may be plotted as a curve to aid in the correlation therebetween. As a result, calibration and sample testing may be conducted under approximately the same conditions at the same time, thus providing reliable quantitative or semi-quantitative results, with increased sensitivity. In the case of a sandwich assay format, the signal provided by the detection working electrode **42** is directly proportional to the analyte concentration in the test sample. In the case of a competitive assay format, which may, for instance,